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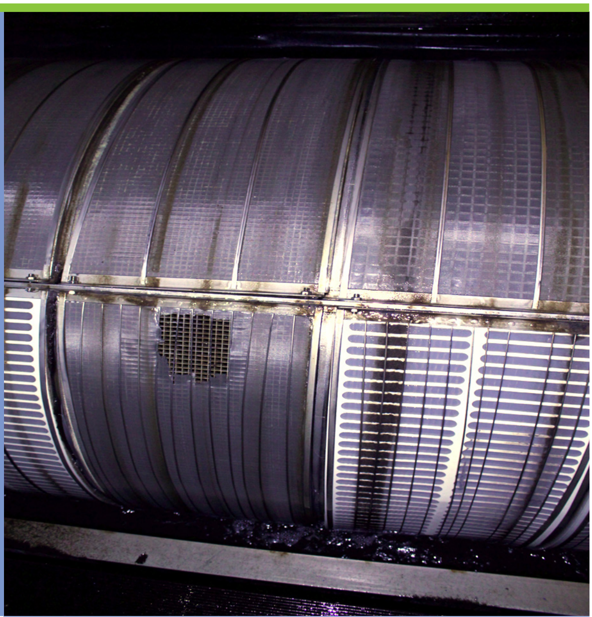
PhD Thesis



Written by Paulo Mira Fernandes
Defended 26 June 2015

DTU Aqua
National Institute of Aquatic Resources

INTERACTIONS BETWEEN MICRO-PARTICLES AND THE REARING ENVIRONMENT IN RECIRCULATING AQUACULTURE SYSTEMS

Ph.D. Thesis by
PAULO MIRA FERNANDES

May 2015

This is a preview. Some pages are omitted from this book.

Section for Aquaculture
National Institute of Aquatic Resources, DTU Aqua
Technical University of Denmark
Hirtshals, Denmark

Colophon

Interactions between micro-particles and the rearing environment in recirculating aquaculture systems

By Paulo Mira Fernandes

Ph.D. thesis

Defended on the 26th of June, 2015

DTU Aqua – National Institute of Aquatic Resources

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Cover photo: Paulo Mira Fernandes (2013)

1. PREFACE

This *Ph.D.* dissertation is submitted in partial fulfilment to attain the Doctor of Philosophy degree (*Ph.D.*). The work shown herein was undertaken during my enrolment as a *Ph.D.* student at the Section for Aquaculture, National Institute of Aquatic Resources (DTU Aqua), Technical University of Denmark, in Hirtshals, Denmark. The research was funded by the Danish Ministry of Higher Education and Science through a Danish innovation consortium titled *Recirculation Technology for Future Aquaculture* (REFA, *Renseteknologier til Fremtidens Akvakultur*).

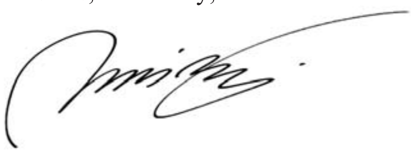
These past few years have been truly overwhelming, shaped by all the people whom I met, and without whom I would not have reached this stage. I could not have found my way without the immeasurable help and inspiration from my two supervisors. My deepest appreciation goes both to Per Bovbjerg Pedersen and Dr. Lars-Flemming Pedersen, whose meticulous approach, valuable insights, and enlightened ideas helped shape some of the most interesting results contained herein.

To Erik Poulsen, Ole M. Larsen, Rasmus F. Jensen, Remko Oosterveld, Dorthe Frandsen, Ulla Sproegel, Brian Møller, and Sara M. Nielsen, goes also a huge thank you: this piece of (my) history could not have been completed without your precious assistance. I would also like to thank the contribution of Dr. Peter V. Skov on the discussion and analysis of potential interactions between particles and fish; and of Carlos Letelier-Gordo on his comments in the first drafts of this thesis. To all my Hirtshals colleagues, heartfelt thanks for the companionship, the long nights discussing hot topics, helping me descend waterfalls, and everything else that cannot be remembered now or described herein. You kept my working insanity sane.

Outside of Hirtshals, I would like to thank all the people that I have met and made me who I am today. A huge thank you goes to, as my father once put it, the other three out of the Fab Four (Tomé, Sara, and Catarina): I would be done to the *beef* without you guys! Nuref, Steffen, Sofie, and all the other 1000 Fryders, thank you all for taking part in my journey.

Last but not least, I would like to express my gratitude to the people who kept me motivated and calm: to my family and friends back *home*, I am glad you always pushed me to keep growing personally and professionally; to Dorthe, thank you for putting up with me and my madness ☺

Hirtshals, 4th of May, 2015



"However, I continue to try and I continue, indefatigably, to reach out. There's no way I can single-handedly save the world or, perhaps even make a perceptible difference – but how ashamed I would be to let a day pass without making one more effort."

Isaac Asimov, 1988
(*The Relativity of Wrong*)

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Paper I: Fernandes, P.M., Pedersen, L.-F., Pedersen, P.B. 2014. Daily micro particle size distribution of an experimental recirculating aquaculture system – A case study. Aquacultural Engineering 60: 28-34. doi:10.1016/j.aquaeng.2014.03.007

Paper II: Fernandes, P.M., Pedersen, L.-F., Pedersen, P.B. 2015. Microscreen effects on water quality in replicated recirculating aquaculture systems. Aquacultural Engineering 65: 17-26. doi:10.1016/j.aquaeng.2014.10.007

Paper III: Fernandes, P.M., Pedersen, L.-F., Pedersen, P.B. 2015. Influence of fixed and moving bed biofilters on micro particle dynamics in an experimental recirculating aquaculture system. Manuscript.

3. LIST OF ABBREVIATIONS

Symbol	**Description**	**Unit**
A_{media}	Total active surface area of media	m^2
AOA	Ammonia oxidizing *Archaea*	-
AOB	Ammonia oxidizing bacteria	-
A:V	Area to volume ratio, as the total surface area divided by the total volume of the fractionated distribution of a particle sample	1/m
BFT	Bio-floc technology	-
BOD_5	Biochemical oxygen demand, after 5 days incubation	mg O_2/L
CFB	Cumulative feed burden, as amount of daily feed delivered per daily volume of make-up water	kg feed/m^3 of make-up water
COD	Chemical oxygen demand of a raw sample	mg O_2/L
C:N	Carbon to nitrogen ratio in the water	-
DBL	Diffusive boundary layer	-
FBB	Fixed bed biofilter	-
FCR	Feed conversion ratio, unit of feed given per unit of weight gain	g/g
FT	Flow-through system	-
HRT	Hydraulic retention time	d
L_{hyd}	Hydraulic loading rate, as water flow rate per cross-sectional area of the filter vessel	$m^3/m^2/d$
MBB	Moving bed biofilter	-
MUW	Make-up water	m^3/d
NOB	Nitrite oxidizing bacteria	-
PSD	Particle size distribution	-
RAS	Recirculating aquaculture system	-
SSA	Specific surface area of biofilter media	m^2/m^3
TAN	Total ammonia-ammonium nitrogen (NH_4^+-N+NH_3-N)	mg N/L
TSS	Total suspended solids	mg/L
WWTP	Wastewater treatment plant	-
β-value	Beta value, as the shape of the particle distribution after applying a double logarithmic transformation	-

4. DANSK RESUMÉ

Fiskeopdræt i recirkulerede systemer (RAS) indebærer en række fordele, en af disse er muligheden for en konstant produktion under stabile forhold året rundt. I modsætning til åbne gennemstrømningsanlæg giver RAS mulighed for optimeret vækst og for reduceret miljøpåvirkning, ligesom f.eks. risikoen for udslip er elimineret. Disse fordele er et resultat af et lukket system med mulighed for kontrol af vandkvalitet på grund af de tilhørende renseforanstaltninger og -komponenter. Disse foranstaltninger er dog endnu ikke fuldt optimerede og især samspillet mellem de enkelte komponenter og deres funktion er af afgørende betydning for optimering af fiskeproduktionen.

De bedste og mest effektive mekaniske rensekomponenter i RAS kan opnå en fjernelsesgrad på op mod 90-95% af det partikulære produktionsbidrag over 30 µm. På den anden side, skaber dette baggrund for en partikelfordeling i anlægsvandet hvor næsten alle partikler er under denne størrelse. Forøget vandskifte er ikke en reel mulighed for at reducere eller kontrollere mikro-partiklernes antal, og de vil derfor typisk have en lang opholdstid i opdrætssystemet.

Udover produktionsbidraget kan partikler også blive genereret i selve systemet, således som det er påvist i såvel adskillige RAS som i gennemstrømningsanlæg. Enhver komponent eller element som skaber turbulens, som f.eks. pumper eller faldende vand, producerer mikro-partikler via nedbrydning af større partikler. Fiskestørrelse og fodersammensætning samt flere andre elementer er ligeledes blevet påvist af have betydende indflydelse på partikler og disses størrelsesfordeling. På den anden side kan eksempelvis biofilter og døde zoner fjerne partikler via aflejring og sedimentation. Det er vigtigt fortsat at identificere komponenternes indflydelse på partikelstørrelsen, således at partikelfjernelsen kan blive yderligere forbedret.

Akkumulering af mikro-partikler kan påvirke fisk og biofilter negativt i RAS. Når partikelstørrelsen mindskes, sker den en relativ øgning i overfladearealet af partiklerne og dermed af kontaktarealet mellem partikler og det omgivende vand. Dette medfører en forøget mulighed for bakterie-vedhæftning og -vækst og dermed også risiko for opformering af potentielt skadelige bakterier, tilstopning af gæller, pumper og filtre samt udvaskning af organisk stof og næringsstoffer fra partiklerne. Udbrud af sygdomme er blevet relateret til akkumulering af partikler i RAS ligesom biofilterfunktionen er påvist at blive reduceret når belastningen med organisk stof medfører, at C:N forholdet overstiger 1:1. Derved reduceres nitrifikationsprocessen i biofilteret idet de nitrificerende bakterier udkonkurreres af de heterotrofe. Dette er påvist for så vidt angår opløst organisk stof, mens betydningen af fin-partikulært materiale endnu ikke er fuld belyst. Formodentlig vil akkumulerede mikro-partikler i biofilteret primært være problematisk under særlige forhold eller driftsbetingelser, men der mangler generelt viden indenfor området.

Denne PhD-afhandling omfatter 3 videnskabelige artikler (2 publicerede og 1 under indsendelse) samt en del ikke-publicerede data fra forskningsarbejdet gennem de sidste 3 år. De tre artikler omhandler: **1**) døgnvariation af mikro-partikler på forskellige steder i RAS, **2**) betydningen af mikrosigte og dennes maskevidde for partikelfordeling og generelle vandkvalitetsparametre i RAS, **3**) produktion eller fjernelse af partikler og organisk stof via biofiltre med bevægeligt henholdsvis fast medie. Studierne blev alle gennemført i veletablerede, modne RAS som blev drevet under konstante betingelser vedrørende indfodring og vandskifte (0.1-3.1 kg/m^3), svarende til normal drift på semi-intensive opdrætsanlæg i Danmark. I alle studier blev effekten af ændringer i system eller opsætning på partikler og partikelfordeling først gennemført når anlæggets baggrunds- eller basisniveauer var konstante og reproducérbare.

I den første artikel (**I**) blev der i et RAS under relativ lav belastning (0.1 kg foder/m^3 vandskifte), undersøgt partikelfordeling (PSD) på en række steder i anlægget gennem 24 timer. Det blev påvist, at partikel-koncentration og -fordeling var stabile og ensartede gennem anlæggets komponenter og -tid. Anlægget var blevet kørt under konstante betingelser og belastning gennem 1 uge forud for prøvetagningen. Overordnet kunne det konstateres, at den relativt lave belastning og et rimeligt internt vandflow på 1,25 gange/time resulterede i en nærmest steady-state situation hvor hverken partikelkoncentrationen eller –fordelingen varierede signifikant mellem de forskellige udtagssteder i anlægget eller tidspunktet på døgnet. Denne steady-state vil være bestemt af drift og design af det enkelte RAS.

I artikel **II** blev betydningen af maskevidden i mikrosigten (100, 60 eller 20 µm) for partikelstørrelsesfordelingen sammenlignet med anlæg uden mikrosigtedug i en triplicat opstilling (12 anlæg i alt). Alle anlæg blev kørt under konstant belastning (3.1 kg foder/m^3 vandskifte) i 6 uger efter at anlæg og biofilter var modne og stabile, hvorefter sigtedug blev installeret. Efter 3 ugers forsøg, begyndte de partikulære faktioner at blive stabile i anlæg med mikrosigte mens de fortsatte med at stige/akkumulere i anlæg uden. Anlæg med 20 og 60 µm nåede ligevægt i uge 3 mens anlæg med 100 µm begyndte at blive stabile i uge 4. Efter 6 ugers drift var betydningen af mikrosigte åbenlys, idet koncentrationen af de partikulære parametre var ca. 30 % mindre end hvad der kunne noteres i anlæg uden mikrosigte. En konstant belastning og replicerede anlæg kørt under ensartede, konstante betingelser med et internt vandflow på 1,75 gange i timen producerede ensartet partikelfordeling i alle anlæg. Partiklerne var alt-overvejende små, mindre end 20 µm, hvilket medvirker til at forklare hvorfor der efter 6 ugers drift ikke var nævneværdig effekt af at anvende en dug på 20 µm i forhold til en på 100 µm.

I den tredje artikel (**III**) blev effekten af fast (fixed-bed) og bevægeligt medie (moving-bed) biofiltre på partikelfordeling og -mængde undersøgt under kontrollerede betingelser og fast belastning (1 kg foder/m^3 vandskifte). Den konstante belastning tillod, at den ene eller den anden type biofilter blev frakoblet systemet og det recirkulerede kredsløb således at effekten af biofiltertype kunne bestemmes, uden at den relative belastning på anlægget fra foder eller fisk blev ændret. Der blev påvist tilbageholdelse af partikler i fixed-bed filtre mens moving-bed filtre forøgede partikelbelastning i systemet, idet større partikeler blev ødelagt og nedbrudt til mindre. Netto-fjernelsen af organisk stof skete med samme rate i begge typer filter, men moving-bed fjernede netto mere af den partikulære fraktion, hvorimod fixed-bed netto fjernede mere af den opløste fraktion. Mekanisk påvirkning af partiklerne via beluftning og bevægelse i moving-bed filterene samt mediets struktur i fixed-bed filtrene er årsag til forskellene i partikler og disses fordeling. Forskellig mikrobiel struktur kan have haft betydning for fjernelsen af organisk stof.

Under forsøgsgangene, som førte til artikel **II** og **III**, blev der også genereret data til undersøgelse af bl.a. **1**) eventuel histo-patologisk effekt af mikro-partikler på regnbueørredernes gæller **2**) sammenhæng mellem mikropartikler og biofilterkinetik og **3**) sammenhæng mellem mikro-partikler og mængde af frit-svømmende bakterier. Der blev ikke påvist nogen oplagte sammenhænge via disse undersøgelser, og derfor er data ikke publiceret her, men de potentielle interaktioner diskuteres desuagtet i kapitler i afhandlingen.

Samlet set ser det således ud til, at partikelparametre opnår en steady-state i RAS under konstante betingelser med ens belasting og vandskifte og med relativt højt internt vandskifte (mindst 1,25 gange/time). Påvirkning af partikelfordelingen kan opnås gennem design og installering af forskellige rensekomponenter, men betydning og interaktion mellem partikler og fisk, partikler og bakterier samt partikler og biofilterfunktion bør undersøges nærmere i fremtidige studier.

5. ENGLISH ABSTRACT

Recirculating aquaculture systems (RAS) have the advantage over other aquaculture systems in terms of stable year-round fish production. Contrary to inland flow through systems or net pen operation, RAS allow for better fish quality and growth, while minimizing the risk of fish escapees. This is derived from the enclosure of the rearing environment, and the installation of water quality control and waste treatment devices. However, waste removal processes are not fully optimized, and the interactions between several of the waste treatment units and their output are of paramount importance for optimal fish growth and performance.

The most efficient solid removal devices in freshwater RAS remove about 90-95% of the solid waste above 30 µm in size. Conversely, this creates background particle distributions comprised mainly of solids with diameters below this range. Since increasing the water exchange rate in RAS may not be a possibility for micro-particle control, this type of particles will often have a long residence time within the system.

Particles can also be produced within the system, as has been identified in several RAS and flow through systems. In general, any element that generates turbulence, such as pumps or waterfalls, produces micro-particles by disintegration of larger particles. Fish size, diet composition, and other farm components, such as degassing units and biofilters, have also been identified as change promoters in particle size or concentration. It is essential to continue identifying components that have an effect on mean particle size, so that solids removal can be further optimized.

Micro-particle accumulation can impair fish and biofilter performance in RAS. As particle size decreases, there is a concomitant increase in the relative contact area between the particle and the water. Practically, this means that there is a greater potential for pathogen adhesion, clogging of fish gills, and leaching of associated nutrients, i.e. organic matter and nitrogen. Fish disease outbreaks have been associated with particle accumulation in RAS, while loading of organic matter on a carbon to nitrogen ratio (C:N) above 1:1 impairs nitrification due to out-competition of nitrifiers by heterotrophic bacteria. This is true for dissolved organic matter; yet, the interactions between biofilters and particulate organic matter in RAS are still not fully described. Presumably, accumulated micro-particles will become a problem only under specific conditions of biofilter size or mode of operation, and fish life stage, although more information is needed on the topic.

The present thesis is accompanied by three scientific articles, and unpublished data acquired during the last three years. The three articles are related to **1)** daily distribution of micro-particles in RAS; **2)** the effects of microscreen mesh size on micro-particles and general water quality parameters in RAS; and **3)** production or removal of particles and organic matter by fixed and moving bed biofilters in RAS. The studies were conducted in matured RAS operated under constant conditions of feeding and make-up water (0.1-3 kg/m^3), reflecting normal operational conditions for semi-intensive RAS in Denmark. In all studies, the effects on particles related to changes in system components or configuration, were only assessed when system background levels demonstrated reproducibility over consecutive sampling dates.

In **paper I**, in a RAS operated at a low cumulative feed burden (CFB) (0.1 kg feed/m^3 make-up water), particle size distribution (PSD) measurements at several locations during a 24-h period, demonstrated the stabilization of particle concentration and distribution parameters. The system was operated under constant conditions of CFB for a week prior to the beginning of the sampling period. Overall, a relatively low feeding level and the internal water turnover rate (1.25 times/h),

steered the PSD towards a quasi-steady-state situation, where neither the concentration, nor the shape of the distribution varied significantly according to sampling location or time of the day.

The effect of mesh size (100, 60 and 20 µm) on PSD, compared to a group without microscreen, was assessed in replicated RAS, as shown in **paper II**. Triplicate RAS for each group were operated under constant CFB conditions (3.1 kg feed/m^3 make-up water) for 6 weeks after the biofilter was mature, as defined by the efficient and constant conversion of ammonia into nitrate. After 3 weeks of operation, solid waste parameters started to stabilize in groups with microscreens, but continued to accumulate in the group without microscreen. The 20 and 60 µm microscreen groups reached equilibrium at week 3, while the 100 µm group started to stabilize after week 4. After 6 weeks of operation, the effect of microscreen presence was apparent, as solid waste parameters were approximately 30 % lower than the waste amounts observed in the group without microscreens. A constant CFB, and replication of the same high internal water turnover rate (1.75 times/h), produced similar PSDs in all systems. These were mostly comprised of micro-particles smaller than 20 µm, which helps explaining why the effect of a 20 µm microscreen was not different from the effect of a 100 µm microscreen after 6 weeks of operation.

In **paper III**, the effects of fixed and moving bed biofilters on PSD were assessed in a RAS operated under controlled CFB (1 kg feed/m^3 make-up water). Particle retention was observed in fixed beds, while moving beds increased the system particle load by disintegration of large particles. Net removal of organic matter occurred at the same rates in both modes of operation, although moving beds removed more of the particulate fraction, and fixed beds removed more of the dissolved fraction. Mechanical stress, induced by aeration in moving beds, and the distribution of the media in fixed beds, caused the observed trends in PSD.

During the experiments related to **paper II** and **paper III**, data was also acquired in order to study the interrelationships between micro-particles and fish gill histopathology; between micro-particles and biofilter kinetics; and between micro-particles and suspended bacteria abundance and activity. There were no clear correlations, and so, the data is not shown in this thesis. Nevertheless, the potential interactions are discussed in detail in specific chapters.

In conclusion, it seems that under constant conditions of feed and make-up water, and an internal water circulation of minimum 1.25 times/h, particulate parameters reach a steady-state. This steady-state is related to system set-up and system operation. Hence, manipulation of the system PSD can be achieved through the installation of different components and devices, such as pumps, mechanical filters or biofilters. The scope of the interactions between particles and fish, bacteria in suspension, or biofilters still needs to be addressed further.

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